

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 29920-76298	FOR FURTHER ACTION <div style="display: flex; justify-content: space-between; font-size: small;"> see Form PCT/ISA/220 as well as, where applicable, item 5 below. </div>	
International application No. PCT/US04/38932	International filing date (<i>day/month/year</i>) 19 November 2004 (19.11.2004)	(Earliest) Priority Date (<i>day/month/year</i>) 20 November 2003 (20.11.2003)
Applicant INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORA		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the Report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. ☐

With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (See Box No. II)

3. ☒ Unity of invention is lacking (See Box No. III)

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

a. the figure of the drawings to be published with the abstract is Figure No. 2



as suggested by the applicant.



as selected by this Authority, because the applicant failed to suggest a figure.



as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

☐

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☐

in written format

☐

in computer readable form

c. time of filing/furnishing

☐

contained in the international application as filed

☐

filed together with the international application in computer readable form

☐

furnished subsequently to this Authority for the purposes of search

2. ☐

In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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Box IV TEXT OF THE ABSTRACT (Continuation of Item 5 of the first sheet)

NEW ABSTRACT

One aspect of the present invention is directed to a dual labeling strategy that enhances the mass spectrometry analysis of peptides, as demonstrated in Figure 2. In one embodiment a *de novo* sequencing method is provided that utilizes both guanidination of lysine residues in conjunction with amidination of the N-termini of peptides to be analyzed by mass spectrometry. This approach facilitates identification of N- and C-terminal fragment ions.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 24/00, 33/00

US CL : 436/173, 86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/173, 86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEARDSLEY et al. "Quantitation using enhanced signal tags: a technique for comparative proteomics", J. Proteome Research, 2003, v. 2, pp. 15-21, published on-line 16 October 2002	1, 4-6, 13-14, 16
X	BRANCIA et al. "Improved matrix-assisted laser desorption/ionization mass spectrometric analysis of tryptic hydrolysates of proteins following guanidination of lysine-containing peptides", Rapid Comm. Mass Spectrom., 2000, v. 14, pp. 2070-2073	1, 4-6, 13-14, 16 ----- 2-3, 15

Y		
X	US 2003/0054570 (QIU et al.) 20 March 2003 (20.03.2003), Abstract, page 1, [0006].	1, 4-6, 13-14, 16 ----- 7-12

Y		

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 March 2005 (22.03.2005)	Date of mailing of the international search report 08 APR 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Yelena G. Gakh, Ph.D. Telephone No. (571) 272-1700 <i>[Signature]</i> Far

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/38932

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions, which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-12, drawn to a method for preparing derivatized peptides.

Group II, claim(s) 13-16, drawn to a set of modified tryptic peptides.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the common technical feature, i.e. a peptide labeled at N-termini with an acetamidine or a propionamidine group, is known in the art (see Bearsley et al., J. Proteome Research, 2003), and therefore is not a special technical feature. This makes the restriction proper.

Continuation of B. FIELDS SEARCHED Item 3:
EAST, STN, Google:

peptide label acetamidine propionamidine MALDI MS

PATENT COOPERATION TREATY

From the

INTERNATIONAL SEARCHING AUTHORITY

DOCKETED

PCT

To:
BRADFORD G. ADDISON
BARNES & THORNBURG LLP
11 SOUTH MERIDIAN STREET
INDIANAPOLIS, IN 46204

FOR Comm. / Report

BY fr

DATE 4/12/05

CHE'D BY _____

DATE _____

WRITTEN OPINION OF THE

INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

08 APR 2005

Applicant's or agent's file reference

29920-76298

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US04/38932

International filing date (day/month/year)

19 November 2004 (19.11.2004)

Priority date (day/month/year)

20 November 2003 (20.11.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): G01N 24/00, 33/00 and US Cl.: 436/173, 86

Applicant

INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORA

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Yelena G. Gakh, Ph.D.

Telephone No. (571) 272-1700

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

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Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ in written format

☐ in computer readable form

c. time of filing/furnishing

☐ contained in international application as filed.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has:
- ☐ paid additional fees
- ☐ paid additional fees under protest
- ☐ not paid additional fees
2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
- ☒ not complied with for the following reasons:

See the lack of unity section of the International Search Report (Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:

- ☒ all parts.
- ☐ the parts relating to claims Nos. _____

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Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims <u>NONE</u>	YES
	Claims <u>1-16</u>	NO
Inventive step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-16</u>	NO
Industrial applicability (IA)	Claims <u>1-16</u>	YES
	Claims <u>NONE</u>	NO

2. Citations and explanations:

Claims 1, 4-6, 13-14 and 16 lack novelty under PCT Article 33(2) as being anticipated by Beardsley et al. (J. Proteome Research, 2003, published on-line October 16, 2002). Beardsley teaches a method for preparing derivatized peptides to enhance mass spectral analysis, MALDI-TOF in particular, comprising dividing a composition comprising peptides into two pools, and labeling one pool with S-methyl thioacetamidine and another pool with S-methyl thiopropioamidine.

Claims 2-3 and 15 lack an inventive step under PCT Article 33(3) as being obvious over Beardsley in view of Brancia et al. (Rapid Comm. Mass Spectr., 2000). Beardsley does not specifically teach blocking lysine residues of the peptide with guanidination using S-methylisothiourea or O-methylisourea, although he indicates that such blocking was performed by others and that it yielded enhanced MALDI-TOF signals. Brancia teaches "improved matrix-assisted laser desorption/ionization mass spectrometric analysis of tryptic hydrolysates of proteins following guanidination of lysine-containing peptides" (Title). It would have been obvious for any person of ordinary skill in the art to combine two chemically compatible methods providing enhanced MALDI-TOF spectra taught by Beardsley and Brancia in order to get combined advantageous of both methods.

Claims 7-12 lack an inventive step under PCT Article 33(3) as being obvious over Beardsley in view of Qiu et al. (US 2003/0054570). Beardsley does not specifically teach isotope substitution of the labeling amidine group. Qiu teaches "isotope-coded ionization enhancement reagents (ICIER)", which upon reaction with proteins give a pool of isotopically labeled proteins; along with isotopically unlabeled proteins this allows comparing MALDI-TOF spectra of two pools. It would have been obvious for any person of ordinary skill in the art to apply Qiu's method involving ICIER to amidine labeling groups disclosed by Beardsley, because this provides the benefits of both methods, i.e. enhancement of MALDI spectra by utilizing amidine labeling disclosed by Beardsley and ICIER enhancement disclosed by Qiu.

Claims 1-16 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 11 and 12 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 11 and 12 are indefinite for the following reason(s): claim 11 recites the limitation "said guanidination", which lacks antecedent basis, as no guanidination is recited in parent claim 10. The same problem of a wrong dependency exists for claim 12.